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(54) Protein preparation in sealed container

(57) A sealed container contains a protein preparation under a pressure of 100-400 mm Hg. The protein preparation can be easily dissolved for administration with little formation of bubbles.

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FIG. 1a



FIG. 1b

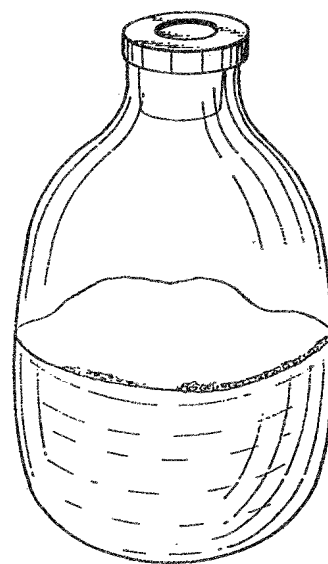


FIG. 2a

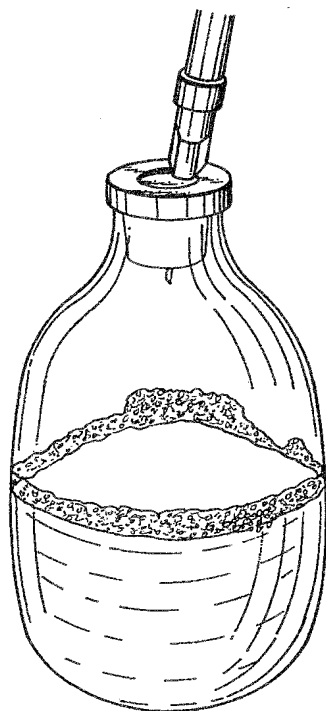
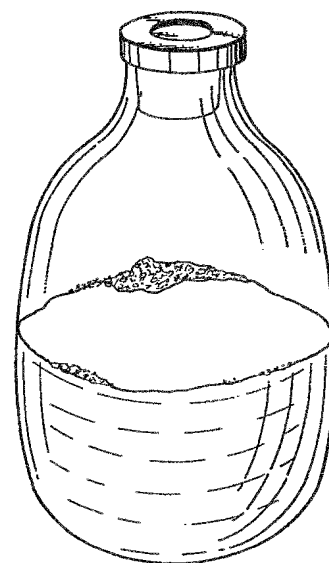


FIG. 2b



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FIG. 3a

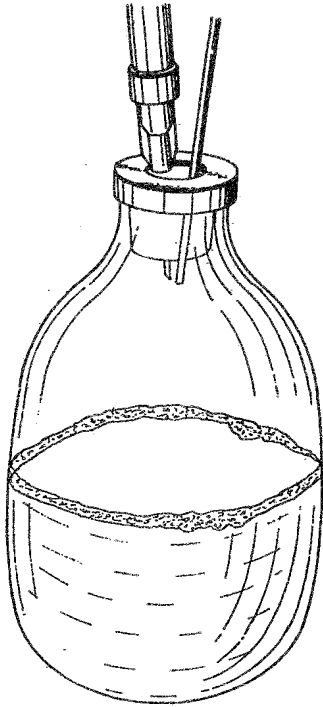


FIG. 3b

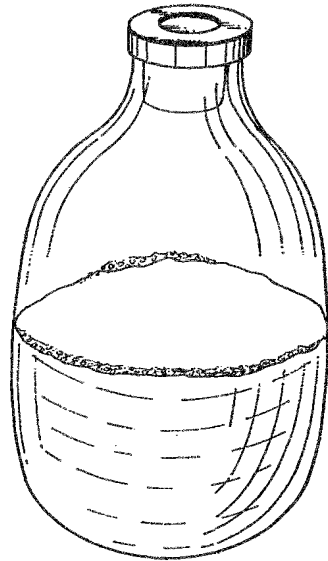
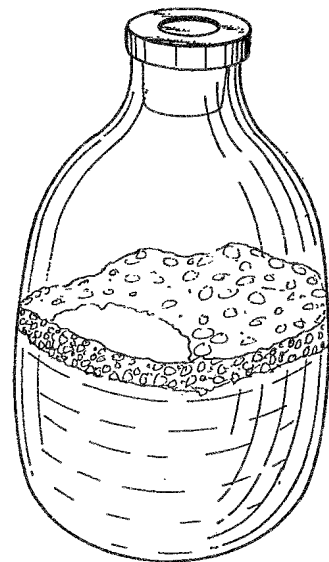


FIG. 4a



FIG. 4b



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FIG. 5a

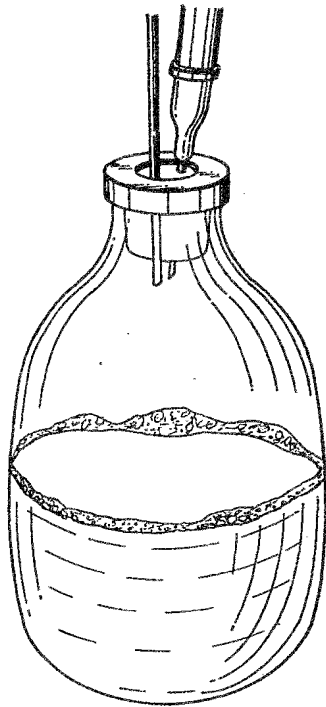
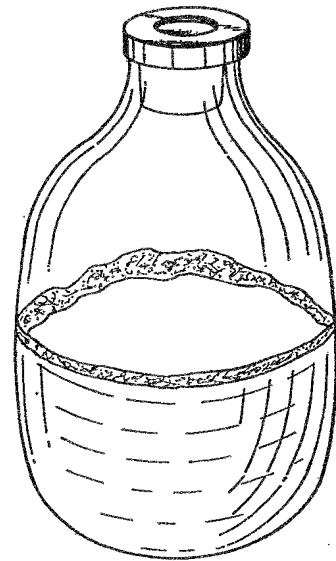


FIG. 5b



## SPECIFICATION

## Protein preparation in sealed container

5 This invention relates to a sealed container containing a protein preparation.

Since protein preparations are unstable in aqueous solution, they are usually prepared and stored as liophilized materials to be dissolved for administration. Protein preparations for example, gamma-globulin or human immunoglobulin prepared by subjecting gamma-globulin to pepsin treatment are usually on sale as diluent injection sets comprising a container of the liophilized medicament, a diluent container, a suction needle, and an injection needle.

Conventional liophilized medicament-containing containers have normal internal pressure or a reduced internal pressure of lower than 3 mm Hg.

For dissolving the medicament in the case of the normal pressure container, there is a system wherein after sucking out air from the container or after inserting an air needle into the container, a diluent is transferred into the container; a hand pump system wherein a hand pump having a suction needle and an injection needle is connected between the medicament-containing container and the diluent container and the diluent is transferred into the container by operating the hand pump; and a gas replacement system wherein the medicament-containing container and the diluent container are connected by two conduits and air in the medicament-containing container is replaced by the diluent. These dissolution operations are complicated, bubbles inevitably form during and remain after transfer of the diluent. Furthermore, if the operator's skill is poor, sparingly soluble masses are liable to form which are difficult to dissolve thereafter.

With the reduced pressure (lower than 3 mm Hg) containers, the dissolution operation is simple, the diluent being automatically transferred into the medicament-containing container on inserting an injection needle connected to the diluent container into the medicament container, but there are such faults that the formation of bubbles during the transfer is severe and bubbles remain in the resulting solution.

It is reported that the formation of bubbles causes the denaturation of proteins (see, "Tanpakushitsu Kagaku (Protein Chemistry)" 3, 523-525 (1973), edited by Shiro Akabori, published by Kyoritsu Shuppan K.K.), so that the formation of bubbles when handling protein preparations is undesirable. For human immunoglobulin such as gamma-globulin, the denaturation of the protein is a particularly serious problem, there being a possibility of condensates forming which are reported to reduce the immunity action of the globulin and cause anaphylaxis shock (see, "J. Immunol", 89, 336-343 (1962) and *ibid.*, 99, 82-91 (1967)).

It is therefore desirable that the formation of bubbles scarcely occurs on dissolution of a protein preparation, particularly a liophilized gamma globulin preparation contained in a sealed container. Further, if many bubbles remain, there is a danger of their

intermixing in a blood vessel and causing a thrombus. Furthermore, the psychological effect of the formation of bubbles and the existence of remaining bubbles on patients and the persons concerned with the patients cannot be disregarded, reducing the commercial value of the medicaments.

According to this invention, there is provided a sealed container containing a protein preparation under a pressure of 100-400 mm Hg, preferably 150-230 mm Hg. For administration, the preparation can be easily dissolved in diluent with the formation and retention of bubbles controlled or prevented.

Figs. 1, 2 and 3 of the accompanying drawings show the bubble formation and retention on dissolving the protein preparations of Examples 1 (170 mm Hg), 2 (190 mm Hg), and 3 (380 mm Hg). Fig. 4 shows such states for the case that the inside pressure of the container was not adjusted, that is, the inside pressure was lower than 3 mm Hg. Fig. 5 shows such states for the case that the inside pressure was normal pressure (760 mm Hg).

The proteins of the protein preparations for this invention may for example be selected from gamma globulin and derivatives thereof. They include various immunoglobulins, such as pepsin-treated human immunoglobulin, plasmin-treated human immunoglobulin, amidated human immunoglobulin, polyethylene glycol-purified human immunoglobulin,  $\beta$ -propiolactone-treated human immunoglobulin, sulfonated human immunoglobulin, hydrochloric acid-treated human immunoglobulin, antichemophilic globulin (human), and other gamma globulins of which a part of the molecule has been removed or substituted, as well as, of course, intact human immunoglobulin.

Furthermore, there may be used such proteins as human serum albumin, human blood plasma, and fibrinogen.

For preparing a sealed container according to this invention, an aqueous solution of a protein may be placed in a container and liophilized, the pressure in the container then being adjusted to 100 to 400 mm.Hg and the container then sealed. Since the liophilized product is amorphous, shows strong hygroscopicity, and frequently becomes unstable on absorption of moisture, it is preferred to adjust the internal pressure by introduction of inert gas into the container and to seal the container, e.g. by a rubber plug.

It is preferred to dissolve the protein preparation by a roller-clamp system as described below but the dissolution may be performed by conventional reduced pressure suction. The roller clamp system employs a tube having a suction needle and an injection needle at its opposite and a roller clamp at its intermediate portion; the injection needle and the suction needle are inserted into the medicament-containing container and a diluent-containing container respectively and the dissolving operation can be performed very simply since when the roller clamp is opened, the diluent in the diluent container is automatically transferred into the medicament-containing container.

The bubble formation and retention on dissolution of protein preparations was compared, using con-

tainers according to this invention and conventional containers, by the following procedure and with the following results.

Experimental procedure:

- 5 Into respective vials each containing liophilized protein preparation a diluent (50 ml of water) was transferred by the respective different procedures indicated below, and then the amount of bubbles formed during the transfer and the amount of bubbles retained after the transfer were measured. The measurement of the amount of retained bubbles was performed as follows; after transferring the

	Protein	Pressure in vial (mm Hg)
25 This invention	(A)*	170
30 Control* <sup>1</sup>	"	<3
Control* <sup>2</sup>	"	normal pressure
35 This invention	(B)*	170
Control* <sup>3</sup>	"	normal pressure
40 This invention	(C)*	170
Control	"	<3
Control	"	normal pressure

45 (A): Dry pepsin-treated human immunoglobulin.  
(B): Plasmin-treated human immunoglobulin.  
(C): Human serum albumin.

\* 1: Globulin for intravenous injection (made by Mochida Seiyaku K. K.)

\* 2: Gamma-Venin (made by Hoechst A. G.)

50 \* 3: Venoglobulin (made by Midorizuyji K. K.)

As is clear from the above table, the control container having normal internal pressure gave extensive bubble formation with great bubble retention, and that having an internal pressure lower than 3 mm Hg showed severe bubbling, while the containers of this invention having properly adjusted internal pressure showed almost no bubbling and much less bubble retention.

The accompanying drawings show the bubble formation and retention on dissolving various protein preparations, Figs. 1a and 1b, 2a and 2b, and 3a and 3b those of Examples 1 (170 mm Hg), 2 (190 mm Hg), and 3 (380 mm Hg) respectively, Figs. 4a and 4b in the case of a container of internal pressure lower than 3 mm Hg, and Figs. 5a and 5b in the case a container whose internal pressure was normal (760 mm Hg).

The (a) Figures show the bubbling directly before finishing the transfer of a diluent (50 ml of water) into the medicament-containing container and the (b) Figures show the bubble retention after shaking the container slowly to dissolve completely the precipitates on its bottom. The dissolutions were performed by a roller-clamp system except in the case of Fig. 5. In the case of Fig. 5, the roller-clamp system

15 diluent into the medicament-containing vial, air in the vial was replaced by helium gas, the bubbles in the solution were broken, and the amount of gas contained in the bubbles was measured by gas chromatography. The results are shown in Table I, wherein the symbols expressing degree of bubble formation are as follows:

- 20 ±: Almost no bubble formation.  
+: Bubbles formed.  
++: Bubbles formed extensively.  
+++: Bubbles formed very extensively.

Table I

Dissolving system	Bubbling formation	Gas volume of bubbles retained (ml)
Roller-clamp system	±	0.2
Reduced press. suction system	+++	0.6
Hand pump system	++	6.0
Roller-clamp system	±	0.3
Gas-replacement system	++	4.8
Roller-clamp system	±	0.2
Reduced press. suction system	++	0.4
Gas-replacement system	+	2.8

could not be employed since the internal pressure of the container was normal pressure and hence a gas replacement system was employed. The pipe disposed on the vial shown in Fig. 3(a) is for adjusting the inside pressure of the container.

As is clear from these Figures, the sealed containers according to this invention, having properly adjusted internal pressure, gives greatly reduced bubble formation and retention as compared with conventional containers.

The invention is further illustrated by the following Examples:

#### Example 1

90 Into a vial was placed 50 ml of an aqueous solution containing 5% dry pepsin-treated human immunoglobulin, 2.25% glycine, and 0.85% sodium chloride and the solution was liophilized. Then, after adjusting the internal pressure of the vial to 170 mm Hg by introducing dry nitrogen gas, the vial was sealed by a rubber plug.

By following the same procedure as in above-mentioned Example 1, sealed vials containing protein preparations under the pressures shown in the following table were prepared

100

Example	Internal pressure of vial
2	190 mm Hg
3	380 mm Hg

5 Sealed vials each containing a medicament different from that (dry pepsin-treated human immunoglobulin) of Example 1 and under a pressure different from that in Example 1, as shown in the following

Example	Medicament	Inside pressure (mm Hg)
4	Plasmin-treated human immunoglobulin	170
5	Amidated human immunoglobulin	100
6	$\beta$ -Propiolactone-treated human immunoglobulin	190
7	Sulfonated human immunoglobulin	160
8	Polyethylene glycol-purified human immunoglobulin*	380
9	Hydrochloric acid-treated human immunoglobulin	160

25 (\*): Purified human immunoglobulin prepared by separating using a fractional precipitation method wherein polyethylene glycol 3000 is added stepwise to human blood plasma according to a standard method.

#### 30 Example 10

Into a vial was placed 50 ml of an aqueous solution containing 5% human serum albumin, 2.25% glycine, and 0.85% sodium chloride and the solution was lyophilized. Then after adjusting the internal pressure of the vial to 170 mm Hg by introducing dry nitrogen gas, the vial was sealed by a rubber plug.

Following the same procedure as in Example 10, sealed vials containing the protein preparation under the pressures shown in the following table were prepared.

Example	Inside pressure of vial
11	190 mm Hg
12	380 mm Hg

#### 45 CLAIMS

1. A sealed container containing a protein preparation under a pressure of 100-400 mm Hg.
2. A container according to claim 1 wherein the preparation is a lyophilized preparation.
3. A container according to claim 1 or 2 wherein the pressure is 150-230 mm Hg.
4. A container according to claim 1, 2 or 3 wherein the pressure is that of inert gas in the container.
5. A container according to any of claims 1 to 4 wherein the protein preparation is a lyophilized gamma globulin preparation.
6. A container according to any of claims 1 to 4 wherein the protein preparation is a lyophilized albumin preparation.
7. A method of preparing a container of protein preparation which comprises disposing the preparation in the container, adjusting the pressure in the container to 100 to 400 mm Hg, and sealing the con-

tainer.

8. A method according to claim 7 wherein the preparation is lyophilized in the container before pressure adjustment.

9. A method according to claim 7 or 8 wherein the pressure is adjusted to 150 to 230 mm Hg.

10. A method according to claim 7, 8 or 9 wherein the pressure is adjusted by introduction of inert gas.

11. A method of preparing a protein preparation for administration which comprises forming a container according to any of claims 1 to 6 and dissolving the preparation in the container by transferring solvent thereinto.

12. A container according to claim 1 substantially as hereinbefore described in any one of Examples 1 to 12.

13. A method of preparing a sealed container of protein preparation, the method being substantially as hereinbefore described in any one of Examples 1 to 12.

14. A method according to claim 11 substantially as hereinbefore described.

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